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RESEARCH ARTICLE

Effects of beneficial microorganisms on lowland rice development

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Abstract Microorganisms can promote plant growth by increasing phytomass production, nutrient uptake, photosynthesis rates, and grain yield, which can result in higher profits for farmers. However, there is limited information available about the physiological characteristics of lowland rice after treatment with beneficial microorganisms in the tropical region. This study aimed to determine the effects of different beneficial microorganisms and various application forms on phytomass production, gas exchange, and nutrient contents in the lowland rice cultivar 'BRS Catiana' in a tropical region. The experiment was performed under greenhouse conditions utilizing a completely randomized design and a 7 × 3 + 1 factorial scheme with four replications. The treatments consisted of seven microorganisms, including the rhizobacterial isolates BRM 32113, BRM 32111, BRM 32114, BRM 32112, BRM 32109, and BRM 32110 and *Trichoderma asperellum* pooled isolates UFRA-06, UFRA-09, UFRA-12, and UFRA-52, which were applied using three different methods (microbiolized seed, microbiolized seed + soil drenched with a microorganism suspension at 7 and 15 days after sowing (DAS), and microbiolized seed + plant spraying with a microorganism suspension at 7 and 15 DAS) with a control (water). The use of microorganisms can provide numerous benefits for rice in terms of crop growth and development. The microorganism types and methods of application positively and differentially affected the physiological characteristics evaluated in the experimental lowland rice plants. Notably, the plants treated with the bioagent BRM 32109 on the seeds and on seeds + soil produced plants with the highest dry matter biomass, gas exchange rate, and N, P, Fe, and Mg uptake. Therefore, our findings indicate strong potential for the use of microorganisms in lowland rice cultivation systems in tropical regions. Currently, an additional field experiment is in its second year to validate the beneficial result reported here and the novel input sustainability.

Keywords *Oryza sativa* · Bioagent · Phytomass yield · Growth promoter · Nutrient uptake · Gas exchange

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Introduction

Rice is one of the most important grains for human consumption (Nascente et al. 2013, 2016). As the human population continues to grow and competition for natural resources increases, there is a consequent need to produce more food in a sustainable way (Clerget et al. 2014). Paddy field ecosystems consist of diverse habitats for different adapted microorganisms over time and space such as aerobic/anaerobic soils, floodwater, rice roots, rice straw stubble, and composted materials (Ahemad and Kibret 2014). Rice producers will need sustainable inputs to meet global feeding and economic demands. There are many new sustainable inputs to promote plant growth, such as organic and silicon fertilization, biochar, and microorganisms. For example, biochar can promote many

beneficial effects on plant growth and its use with microorganisms could produce interactive effects on plant development (Novotny et al. 2015; Nadeem et al. 2017.

In this sense, the use of microorganisms that can promote beneficial effects on plant and crop growth may be a good strategy for sustainable agriculture and may explain why the practice is increasing in crop systems worldwide (Isawa et al. 2010). Among the existing bacteria known to interact with plants, plant growth-promoting rhizobacteria (PGPR) interact with plant root and can promote plant development. The effects of PGPR can be direct, such as hormone and siderophore production, phosphate solubilization, nutrient mobilization, and liberation of enzymes such as lipases and protease, or indirect by suppressing various pathogens in the forms of biocontrol agents (Ahemad and Kibret 2014) and by providing greater efficiency in the gas exchange process (Nascente et al. 2017).

In this sense, another important group of microorganisms acting as biofertilizer producers is *Trichoderma* spp., a fungal species present in nearly all soils. *Trichoderma* spp. have been known for decades to increase plant growth and crop yield (Lindsey and Baker 1967; Chang et al. 1986), to improve crop nutrition and fertilizer uptake (Harman 2000; Yedidia et al. 2001), to speed up plant growth and enhance plant greenness (Harman 2006), and to control numerous plant pathogens (Cuevas et al. 2005; Shoresh and Harman 2008). Some of these effects may be associated with the apparent ability of *Trichoderma* spp. to hasten the mineralization of organic materials (Cuevas 1991), which likely causes the release of nutrients from soil organic matter.

In addition, these microorganisms may provide protection against biotic stress, such as through the induction of resistance and direct antagonism (Spaepen et al. 2009). The biological control of soil-borne pathogens is often attributed to improving the nutrition that boosts host defenses or directly inhibits pathogen activity and growth (Ahemad and Kibret 2014). In addition, antagonistic microorganisms, e.g., *Pseudomonas* spp., *Bacillus* spp. (Wiwattanapatapee et al. 2007), *Burkholderia* spp. (Cuong et al. 2011), and *Trichoderma* spp. (Khan and Sinha 2006), have been used to control disease.

Many PGPM (plant growth-promoting microorganisms) are being investigated for their roles as plant growth promoters in rice plants, such as *Azospirillum* strain AbV5 (Isawa et al. 2010), *Gluconacetobacter* (Muthukumarasamy et al. 2005), *Herbaspirillum*, *Burkholderia* (Baldani et al. 2000), *Pseudomonas* (Yao et al. 2010), *Bacillus*, *Serratia*, *Paenibacillum*, *Enterobacter*, and *Klebsiella* (Spaepen et al. 2009). Studies performed at Embrapa Rice and Beans selected promising rhizobacteria isolates (BRM 32113, BRM 32111, BRM 32114, BRM 32112, BRM 32109, and BRM 32110) that provided increased crop biomass production, nutrient uptake, and disease resistance in upland rice (Filippi et al. 2011; Silva et al. 2012). Additionally, during studies conducted at the Federal Rural University of Amazon, four isolates of *Trichoderma asperellum* were selected and tested as growth promoters and biocontrol agents both in the greenhouse and in the field (França et al. 2015).

However, there is still a lack of information about the role of these bioagents (rhizobacteria and *T. asperellum*) on gas exchange and nutrition of rice plants in tropical region floodplains. Further, for many crops, positive results such as higher grain yield or plant biomass production were observed when in association with PGPM, including sugarcane (Lopes 2000), *Zea mays* (Dartora et al. 2013), *Phaseolus vulgaris* (Martins 2013), *Eucalyptus grandis* (Moreira and Araújo 2013), and *Oryza sativa* (Souza-Junior et al. 2010). However, the successful utilization of PGPM is strongly dependent on its survival in the soil, compatibility with the host crop, the ability to interact with indigenous soil microflora, and other environmental factors (Vejan et al. 2016).

The hypothesis of this study is that the previously selected bioagents for rice, when applied via either seed or soil/foliar spray, will significantly affect the growth of lowland rice plants. Therefore, this study aimed to determine the effect of the selected microorganisms and methods of application on the biomass production, gas exchange, and nutrient content in lowland rice plants of the 'BRS Catiana' cultivar in a tropical region.

Materials and methods

Environmental characterization

The experiments were conducted at the Capivara Experimental Station of Embrapa Rice and Beans located at Santo Antônio de Goiás, GO, Brazil (16°28′00″S and 49°17′00″W). Elevation at the site is 823 m. The climate is classified as tropical savanna (Aw according to its Köppen classification). There are two well-defined seasons: a dry season from May to September (autumn/winter) and a wet season from October to April (spring/summer). The average annual rainfall is between 1500 and 1700 mm, and the average annual temperature is 22.7 °C and ranges annually from 14.2 to 34.8 °C. The experiment was conducted in a greenhouse using soil from the arable layer (0–0.20 m) of an Inceptisol (clay-loamy, isothermic, mesic Typic Haplaquepts). The chemical characteristics of the soil were determined according to the methods described by Claessen (1997). The results were as follows: pH (H_2O) = 6.0; hydrolytic acidity = 44.2 $mmol_c\ kg^{-1}$; Ca^{2+} = 56.0 $mmol_c\ kg^{-1}$; Mg^{2+} = 23.5 $mmol_c\ kg^{-1}$; Al^{3+} = 0 $mmol_c\ kg^{-1}$; $H^+ + Al^{3+}$ = 11.0 $mmol_c\ kg^{-1}$; P = 52.2 mg kg^{-1}; K^+ = 125 mg kg^{-1}; Cu^{2+} = 1.2 mg kg^{-1}; Zn^{2+} = 4.4 mg kg^{-1}; Fe^{3+} = 30.6 mg kg^{-1}; Mn^{2+} = 38.7 mg kg^{-1}; total exchangeable

bases = 82.70 $mmol_c$ kg^{-1}, soil exchangeable cations = 93.70 $mmol_c$ kg^{-1}; base saturation = 88.26%, total N = 1.27 g kg^{-1}, total C = 1.27 g kg^{-1}, and soil organic matter = 25.37 g kg^{-1}. The soil texture shows a content of 331 g kg^{-1} of clay, 246 g kg^{-1} of silt, and 423 g kg^{-1} of sand. The soil bulk density was 0.96 Mg m^{-3}.

Three weeks before rice planting, the 7-kg pots were completely filled with the soil and fertilized with 67.2 mg kg^{-1} of N (urea), 167.68 mg kg^{-1} P (simple superphosphate), and 159.39 mg kg^{-1} of K (potassium chloride). Soil moisture was monitored daily by weighing the pots until N topdress fertilization (20 days after rice emergence) throughout the experiment. The evapotranspirated moisture was replaced when it reached 85%, increasing it to 100% of the field capacity, and the water level was maintained 3 cm above the soil.

Experimental design and treatments

The experimental design was completely randomized in a 7 × 3 + 1 factorial, with four replications. The treatments consisted of seven microorganisms, six rhizobacterial isolates, and a *Trichoderma asperellum* pool with three application forms. The rhizobacterial isolates (BRM 32113, BRM 32111, BRM 32114, BRM 32112, BRM 32109, and BRM 32110) are described in Table 1, and they are currently stored and preserved in the Multifunction Microorganisms and Fungi Collection from Embrapa Rice and Beans. The *T. asperellum* pool was composed of four isolates, UFRA-06, UFRA-09, UFRA-12, and UFRA-52, which were isolated from rhizospheric soils of reforested and native forest areas in the Amazon and taxonomically identified by Silva et al. (2012) and are currently stored and preserved in the Fungal Culture Collection of the Plant Protection Laboratory at the Federal University Rural of Amazon. The three application forms used were as follows: 1 seed - microbiolized seeds; 2 seeds - soil - microbiolized seeds + soil drenched with microorganism suspension at 7 and 15 days after sowing (DAS); and 3 seeds - plant - microbiolized seeds + plant spraying with microorganism suspension at 7 and 15 DAS. The control treatment consisted of no microbiolized seed, soil drenched with only water, and plants sprayed with only water (i.e., without any microorganisms).

Seed microbiolization

The bacterial suspensions were prepared with water from cultures that had been growing for a 24-h period on solid medium 523 (Kado and Heskett 1970) at 28 °C, and the concentration was determined using a spectrophotometer until A540 = 0.5 (10^8 UFC). The rice seeds were immersed in each suspension, and the control seeds were immersed in water for a period of 24 h under constant agitation at 25 °C.

Each isolate of the *T. asperellum* pool was grown in a Petri dish containing PDA (potato dextrose and agar) for 5 days and bioformulated as described by Silva et al. (2012). The seed treatment was performed at concentrations of 10 g of powdered (Silva et al. 2012) *T. asperellum* per 1 kg of seed (Filippi et al. 2011; Silva et al. 2012). The concentration of the biological suspension was 10^8 conidia ml^{-1}.

- Soil drench: 100 ml of suspension of each treatment, all the bacterial isolates (10^8 CFU), the *T. asperellum* pool (10^8 conidia ml^{-1}), and water drenched the trial soil at 7 and 15 DAS.
- Plant spray pulverization: 30 ml of suspension of each treatment, all the bacterial isolates (10^8 CFU), the *T. asperellum* pool (10^8 conidia ml^{-1}), and water were sprayed on the plants using a manual backpack sprayer

Table 1 Collection code, origin, biochemical characteristics, and taxonomic classification of the six rhizobacterial isolates utilized for seed and plant treatments

Code[a]	Origin[b]	Color[c]	Biochemistry[d]					Taxonomy[e]
			AIA[f]	Celul.[g]	Phosf.[h]	Sider.[i]	Biofilm[j]	
BRM 32111 (R-55)	PA/Brazil	Yellow		+	+	+	+	*Pseudomonas* sp.
BRM 32113 (R-46)	PA/Brazil	Pink	+	+		+	+	*Burkholderia* sp.
BRM 32114 (R-235)	PA/Brazil	Pink	+	+	+	+	+	*Serratia* sp.
BRM 32112 (20.7)	GO/Brazil	Yellow		+	+	+	+	*Pseudomonas* sp.
BRM 32109 (82)	GO/Brazil	White		+	+	+	+	*Bacillus* sp.
BRM 32110 (138)	PA/Brazil	White		+	+		+	*Bacillus* sp.

[a] Number code of rhizobacterial isolates in the Microorganisms and Fungi Multifunction Embrapa Rice and Beans Collection

[b] Geographical origin of each isolate

[c, d, e] Colony color, biochemical characterization, and taxonomic classification of each isolate, described by Martins (2015)

[f, g, h, i, j] Indoleacetic acid producer, cellulase producer, phosphatase producer, siderophore producer, and biofilm producer. The methodology is described in Martins (2015)

[+] Plus signs means that the rhizobacteria present the biochemistry effect (AIA, Celul., Phosf., Sider. or Biofilm)

at a constant pressure provided by a CO_2 pressure source and a conical nozzle type (TX-VS2) at 7 and 15 DAS.

Management of rice plants

Ten rice seeds were sown per pot (BRS Catiana cultivar) on July 13, 2016. The plant emerged on July 19, 2016. Ten days after germination, we thinned the shoots and kept three plants per pot. At the beginning of the rice tillering stage (August 2, 2016), topdressing fertilization (1 g of ammonium sulfate per pot) was performed, and another topdressing with the same amount (1 g of ammonium sulfate per pot) was performed at the middle tillering stage (August 12, 2016). Weeds were controlled manually and there was no incidence of insects or diseases.

Gas exchange

The leaf gas exchange parameters and sheet width, LW (cm), were sampled from the lowland rice plants. We measured the following parameters: photosynthetic rate, *A* (μmol CO_2 m^{-2} s^{-1}), transpiration rate, *E* (mmol H_2O m^{-2} s^{-1}), stomatal conductance, gs (mol H_2O m^{-2} s^{-1}), internal CO_2 concentration, Ci (vpm), and leaf temperature, Tleaf (°C), determined using a portable gas meter in the infrared region of IRGA (LCpro + ADC BioScientific) from 08:00 to 10:00 am, at 33, 71, and 101 DAE (days after emergence).

Gas exchange in the youngest fully expanded leaves was measured during the first two evaluation periods and on the flag leaf in the last evaluation. The equipment was set to use concentrations of 370–400 mol mol^{-1} CO_2 in the air, which is the reference condition used in the IRGA photosynthesis chamber. The photosynthetic photon flux density (PPFD) used was 1200 μmol [quanta] m^{-2} s^{-1}. The minimum equilibration time for performing the reading was 2 min.

Biomass production

Rice shoots from all plants in each pod were collected for biomass assessment 101 days after seed emergence when 50% of plants were in full flower. The plants from each treatment were dried at 65 °C until at a constant weight and weighed to determine the dry matter of the biomass.

Concentrations of nutrients in rice plants

Following the drying and weighing of the collected plants, aliquots were taken and ground, and analysis of the nutrient content (N, P, K, Ca, Mg, Fe, Zn, Cu, and Mn) was performed with the shoots in accordance with recommendations by Malavolta et al. (1997).

Statistical analysis

All data were analyzed using analysis of variance and the *F* probability test. Microorganism species and application form were considered as fixed effects. Blocks and all block interactions were considered random effects. A comparison of means was performed with Tukey's test ($p \leq 0.05$). Dunnett's test was performed with a significance threshold of $p \leq 0.05$ to compare the treatment without microorganisms (control) with each treatment with microorganism application. We used the SAS statistical package (SAS 1999).

Results

Leaf gas exchange evaluation

The photosynthetic rate (*A*) was significantly higher when lowland rice plants were treated with rhizobacteria BRM 32110 (14%), BRM 32112 (12%), BRM 32113 (10%), and BRM 32109 (10%) and *T. asperellum* pool (9%) compared to the control (Table 2). The transpiration rate (*E*) values of rice plants treated with microorganisms were similar to those of the control, except for BRM 32110. The stomatal conductance (g*s*) of rice plants treated with the BRM 32109 isolate presented the highest value (13%) and differed statistically from the control treatment. There were no significant differences in internal CO_2 concentration (Ci) and leaf temperature (Tleaf) among plants treated with microbial isolates and the control. The three different microorganism application methods did not affect *A*, gs, Ci, or Tleaf. However, they affected *E*, which was higher in the microbiolized seed + drenched soil and microbiolized seed + sprayed plant treatments, and both treatments significantly differed from the microbiolized seed treatment. In terms of evaluation time, the lowland rice plants had increased leaf width (LW), the *A* and gs values from the first (33 DAE) to the last evaluation (101 DAE). The maximum value of *E* was observed at 71 DAE and of Ci was observed at 101 DAE. The interaction between bioagents and application forms was significant for *E*, gs, and Ci (Table 3). For the photosynthetic rate, the isolate BRM 32109 produced among the highest *E*, gs, and Ci in treated plants at both evaluation periods.

Shoot dry biomass

There were only single effects of the bioagent addition and application forms for shoot phytomass production, with no significant interactions (Table 4). Isolate BRM 32109 produced the largest increase (49.31 g) and differed from the control (40.47 g). The remaining isolates did not produce significant differences in biomass production compared to the control treatment. Isolate BRM 32109 also differed in its

Table 2 Microorganism types and application method effects on leaf width (LW), photosynthesis (*A*), transpiration (*E*), stomatal conductance (gs), internal CO_2 concentration (Ci), and leaf temperature (Tleaf) of lowland rice plants at three evaluation times: 33, 71, and 101 DAE (days after emergence); and ANOVA results (*F* probability)

Factors	LW	*A*	*E*	gs	Ci	Tleaf
Microorganism	cm	μmol CO_2 m^{-2} s^{-1}	mmol H_2O m^{-2} s^{-1}	mol H_2O m^{-2} s^{-1}	vpm	°C
BRM 32113 (R-46)	1.24 ab[§]	18.19 ab*[§§]	8.20 a	0.94 abc	328 ab	29.10
BRM 32112 (R-55)	1.26 ab	17.47 bc	7.59 a	0.99 ab	331 ab	28.90
BRM 32114 (R-235)	1.22 b	16.77 c	7.72 a	0.92 bc	335 a	29.20
BRM 32111 (20.7)	1.23 ab	18.47 a*	7.69 a	0.86 c	330 ab	29.07
BRM 32109 (82)	1.28 a	18.01 ab*	7.95 a	1.02 a*	329 ab	29.44
BRM 32110 (138)	1.27 ab	18.56 a*	6.93 b	0.98 ab	327 b	28.77
T. asperellum pool	1.27 ab	17.86 ab	7.76 a	0.95 ab	333 ab	28.61
Control[§§§]	1.26	16.33	7.88	0.90	332	28.98
Application form						
Seed	1.24	17.55	7.36 b	0.94	330	29.08
Seed-soil	1.27	18.13	7.74 ab	0.94	329	29.18
Seed-plant	1.24	17.81	8.06 a	0.94	333	28.84
Evaluation time						
33 DAS	0.83 c	15.05 c	7.86 b	0.42 c	330 b	29.72 a
73 DAS	1.43 b	18.54 b	8.90 a	0.96 b	326 b	29.87 a
101 DAS	1.50 a	20.13 a	6.31 c	1.48 a	337 a	27.44 b
Factors	ANOVA (*F* probability)					
Microorganism (M)	0.0316	0.0048	0.0101	0.0125	0.0417	0.8592
Application form (F)	0.1310	0.2021	0.0106	0.9415	0.1434	0.4858
Evaluation time (E)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
M × F	0.6174	0.0891	0.4104	0.0991	0.0697	0.9488
M × E	0.4805	0.2209	0.0002	< 0.001	0.0313	0.8057
F × E	0.2077	0.0601	0.0599	0.0602	0.3214	0.3866
M × F × E	0.4473	0.2556	0.4478	0.0527	0.0968	0.7964

[§] Means followed by the same letter do not differ by Tukey test at $p \leq 0.05$. [§§] Means followed by '*' differ from the control treatment (no microorganism) by the Dunnett Test at $p \leq 0.05$. [§§§] Control means no bioagent treatment

effects on biomass production from BRM 32113 (42.06 g), BRM 32111 (41.76 g), BRM 32114 (41.57 g), BRM 32110 (42.61 g), and BRM 32110 (41.93 g). The application of BRM 32109 in the seed-soil resulted in the highest biomass production (46.27 g), differed from the treatment with application on seeds and plants (41.64 g), and was similar to the treatment with application only on seeds (42.88 g).

Nutrient content in rice plants

Nutrient uptake was affected by the different bioagent treatments. Rice plants treated with BRM 32113, BRM 32114, BRM 32109, and BRM 32111 showed significant increases in N uptake (Table 5). The isolate BRM 32110 produced the highest level of K in the rice shoots. The iron content in the rice shoots was affected only by BRM 32109 and the *T. asperellum* pool. The rhizobacteria BRM 32113, BRM 32109, and BRM 32110 produced the highest values of Mn in rice plants. Regarding zinc content in rice plants, addition of the rhizobacterium BRM 32112 produced the highest value and differed from the other isolates. There was no effect of microorganism treatment on P, Ca, Mg, and Cu, and there was no effect of the different application forms on P, K, Ca, Mg, and Cu uptake by shoots of rice plants (Table 5). There was no interaction between the bioagent and application forms for any of the nutrients evaluated.

The N content was higher in shoot plants in the seeds and seed-soil and was significantly different from the seed-plant application (Table 5). The iron and Mn contents in rice shoots were higher after the application of microorganisms only in the seeds, and this treatment differed from the other forms of application. The highest Zn contents were obtained when bioagents were applied to seeds and soil.

The N uptake by lowland rice plants coexisting with the rhizobacteria BRM 32113, BRM 32114, BRM 32111, and BRM 32109 and *T. asperellum* pool differed from the control (Table 5). The P content was higher when rice was treated with the rhizobacterium BRM 32109. The K content was

Table 3 Interactions between microorganism types and evaluation times for transpiration (*E*), stomatal conductance (gs), and internal CO_2 concentration (Ci) of lowland rice plants in three evaluation times: 33, 71, and 101 DAE (days after emergence)

Evaluation time at 33 DAE			
Factors	*E*	gs	Ci
Microorganism	mmol H_2O m^{-2} s^{-1}	mol H_2O m^{-2} s^{-1}	vpm
BRM 32113 (R-46)	9.49 a§	0.44	322 b
BRM 32112 (R-55)	7.58 ab	0.41	328 ab
BRM 32114 (R-235)	8.04 a	0.40	345 a
BRM 32111 (20.7)	8.26 a	0.47	327 ab
BRM 32109 (82)	8.13 a	0.45	332 ab
BRM 32110 (138)	5.69 b	0.38	325 ab
T. asperellum pool	7.86 a	0.38	329 ab
Evaluation time at 71 DAE			
Factors	*E*	gs	Ci
Microorganism	mmol H_2O m^{-2} s^{-1}	mol H_2O m^{-2} s^{-1}	vpm
BRM 32113 (R-46)	8.64 ab	0.82 c	328 abc
BRM 32112 (R-55)	8.73 ab	1.09 a	323 abc
BRM 32114 (R-235)	9.04 ab	1.01 ab	318 c
BRM 32111 (20.7)	9.34 a	0.91 bc	324 abc
BRM 32109 (82)	9.21 ab	1.04 ab	321 abc
BRM 32110 (138)	8.45 b	0.93 bc	330 ab
T. asperellum pool	8.88 ab	0.93 bc	332 a
Evaluation time at 101 DAE			
Factors	*E*	gs	Ci
Microorganism	mmol H_2O m^{-2} s^{-1}	mol H_2O m^{-2} s^{-1}	vpm
BRM 32113 (R-46)	6.48 a	1.56 ab	335 ab
BRM 32112 (R-55)	6.46 a	1.50 ab	343 a
BRM 32114 (R-235)	6.08 ab	1.36 bc	339 ab
BRM 32111 (20.7)	5.49 b	1.21 c	340 ab
BRM 32109 (82)	6.50 a	1.55 ab	334 ab
BRM 32110 (138)	6.65 a	1.65 a	327 b
T. asperellum pool	6.52 a	1.54 ab	338 ab

DAE days after rice emergence

§ Means followed by the same letter do not differ by Tukey test at $p \leq 0.05$

Table 4 Effects of microorganism type and application method on biomass dry matter of rice shoots and ANOVA results (*F* probability)

Factors	Shoot biomass
Microorganism	Grams
BRM 32113 (R-46)	42.08 b§
BRM 32112 (R-55)	41.76 b
BRM 32114 (R-235)	41.57 b
BRM 32111 (20.7)	42.61 b
BRM 32109 (82)	49.31 a*
BRM 32110 (138)	41.93 b
T. asperellum pool	45.93 ab
Control§§§	40.47
Application form	
Seed	42.88 ab
Seed-soil	46.27 a
Seed-plant	41.64 b
Factors	ANOVA (*F* probability)
Microorganism (M)	0.0232
Application form (F)	0.0123
M × F	0.4094

§ Means followed by the same letter do not differ by Tukey test at $p \leq 0.05$. §§ Means followed by '*' differ from the control treatment (no microorganism) according to the Dunnett Test at $p \leq 0.05$. §§§ Control means no bioagent treatment

higher when rice was treated with the rhizobacterium BRM 32110. The calcium, Mg, and Cu contents in the rice plants did not differ from those of the control treatment. The level of Fe was higher in rice shoots treated by the bioagents BRM 32113, BRM 32112, BRM 32109, and *T. asperellum* pool. The manganese content was higher in rice plants when treated with the bioagents BRM 32113, BRM 32111, and BRM 32110. The levels of Zn were higher in rice plants when treated with the rhizobacteria BRM 32112, BRM 32109, and BRM 32110 and *T. asperellum* pool.

And finally, BRM 32109 increased the contents of N, P, Fe, and Zn; BRM 32110 increased the contents of K, Mn, and Zn; BRM 32111 increased N, Mn, and Zn; BRM 21112 increased the contents of Fe; BRM 32113 increased the contents of N, Fe, and Mn; and BRM 32114 increased the content of N.

Discussion

Irrigated rice cultivated in tropical regions is under favorable climatic conditions for the development of diseases and insect attacks (Santos et al. 2006). To achieve satisfactory yield, these crops require management consisting of voluminous applications of materials such as nitrogen fertilizers, herbicides, insecticides, and fungicides.

Currently, these applications are calendar-based and disregard the nutritional and phytosanitary status of the crop (Santos et al. 2006). This practice has threatened the quality of water, fauna, and local flora and poses a risk of contamination for farmers and consumers. In this scenario, the insertion of multifunctional bioagents for management of tropical irrigated rice crop represents an important sustainable practice due to the reduction of petroleum-derived inputs, insecticides, and chemical fungicides. The same bioagents used in this study were selected and characterized as growth promoters and promising biological agents for the control of leaf blast (*Magnaporthe oryzae*) and sheath blight (*Rhizoctonia solani*). This multifunctionality led us to investigate additional aspects of the interaction between these isolates and irrigated rice plants.

Table 5 Microorganism application form effects on nutrient content in rice shoots and ANOVA results (*F* probability)

Factors	N	P	K	Ca	Mg	Cu	Fe	Mn	Zn
Microorganism	g kg^{-1}					mg kg^{-1}			
BRM 32113 (R-46)	31.12 a*[§]	4.14	26.46 a	9.02	4.36	8.47	164 b*	1652 a*	32.83 c
BRM 32112 (R-55)	25.88 c	4.58	22.75 d	7.46	4.17	6.97	165 b*	1375 c	39.13 a*
BRM 32114 (R-235)	30.92 ab*	4.37	25.84 ab	7.25	4.06	6.45	152 c	1334 c	31.85 cd
BRM 32111 (20.7)	28.46 ab*	4.07	23.21 cd	8.18	4.09	6.29	152 c	1484 b*	30.15 d
BRM 32109 (82)	29.09 ab*	5.40*	24.98 bc	8.65	4.26	6.61	183 a*	1620 a*	35.97 b*
BRM 32110 (138)	25.97 c	4.56	28.20 a*	7.58	3.62	7.95	146 c	1549 ab*	35.31 b*
T. asperellum pool	28.91 b*	4.40	23.82 cd	7.02	3.75	6.66	184 a*	1386 c	36.59 b*
Control	24.84	3.51	24.40	8.66	3.32	6.39	142	1338	30.06
Application form									
Seed	29.96 a	4.47	25.32	8.41	4.26	7.73	181 a	1657 a	34.55 b
Seed-soil	30.37 a	4.90	24.90	7.42	3.84	7.24	150 b	1413 b	36.24 a
Seed-plant	25.54 b	4.14	24.89	7.80	4.04	6.20	160 b	1387 b	32.84 c
Factors	ANOVA (*F* probability)								
Microorganism (M)	< 0.001	0.5613	< 0.001	0.2308	0.7156	0.0541	< 0.001	< 0.001	< 0.001
Application form (F)	< 0.001	0.8803	0.0587	0.2479	0.9821	0.1782	0.0314	0.0137	0.0004
M × F	0.2146	0.9668	0.2196	0.0528	0.9907	0.0682	0.3716	0.2417	0.0874

[§] Means followed by the same letter do not differ by Tukey test. [§§] Means followed by '*' differ from the control treatment (no microorganism) by the Dunnett Test at $p \leq 0.05$. [§§§] Control means no bioagent treatment

Understanding the physiological changes that occur in plants treated with bioagents can help to predict their positive effects on crop growth and grain yield. Thus, this study tested the interaction of the lowland rice genotype (BRS Catiana cultivar) and different beneficial microorganisms, and the results showed an increase in photosynthesis (*A*) rates. This is important because photosynthesis is a major driver of crop yield and the key process affected by this system (i.e., the presence of bioagents in lowland rice cultivation) (Table 2).

The leaves of lowland rice plants treated with BRM 32113, BRM 32110, BRM 32112, BRM 32109, and *T. asperellum* pool displayed larger *A*, but only plants treated with the BRM 32109 isolate displayed larger stomatal conductance (gs). Similar results from *A* were found for well-watered upland and lowland rice plants in a study by Centritto et al. (2009). These increases were coupled with one of the highest transpiration rates (*E*) (i.e., except for BRM 32110). This suggests that the BRM 32110 isolate can promote more efficient functionality in the biochemical machinery, since the photosynthetic CO_2 utilization was greater compared to that of the other treatments. Several studies of the beneficial effects of PGPMs in plants have reported that the phytomass increase may be due to the higher photosynthetic rates. According to Thakur et al. (2010) and Poupin et al. (2013), the higher photosynthetic rate in rice plants is a result of the increased efficiency of photochemical machinery, while (Shoresh and Harman 2008). Naveed et al. (2014), and Segarra et al. (2007) report that it may be due to the increased expression of Rubisco, Rubisco activase, and proteins from photosystem II genes.

Application of the rhizobacterium BRM 32109 resulted in the highest dry shoot phytomass of the rice plants (Table 4). This could be related to the increase in *A* and gs and the uptake of some nutrients. In addition, rice plants treated with this rhizobacterium differed from the control treatment in N, P, Fe, Mn, and Zn. This is a notable result, as flooded soils are known to have lower concentrations and availability of Zn. This deficiency can be corrected by using bioagents that increase the bioavailability of Zn from various forms such as $Zn_3(PO_4)_2$, $ZnCO_3$, and ZnO (Fageria 1984; Shakeel et al. 2015). Our results suggest that increased nutrient content in lowland rice plants may have produced a direct effect on phytomass production, which in turn has a significant effect on crop yield (Fageria 2009; Fageria et al. 2011). Although the isolates BRM 32113 and BRM 32114 are AIA producers (Table 1), bioagents can provide indirect plant growth stimulation by improving the availability and absorption of nutrients (Pérez-García et al. 2011; Zhang et al. 2011). Nascente et al. (2017), working with the same bioagents in upland rice, reported that the rhizobacterium BRM 32114 promoted increases in physiological characteristics, biomass production, and nutrient uptake. Likewise, Rego et al. (2014) reported that rice seeds treated with the *T. asperellum* pool, BRM 32113, and BRM 32111 showed changes in root architecture and confirmed that treated plants were more efficient in nutrient uptake.

The use of bioagents to improve plant development is becoming increasingly common worldwide (Ahemad and Kibret 2014; Nascente et al. 2017). However, evaluations of these bioagents under field conditions are rare, probably based on

previous results by others showing that bioagent products present problems with instability (Shakeel et al. 2015). This is one of the reasons that we chose to compare three different application methods and selected bioagents from the rhizosphere and phyllosphere of rice.

This paper presents a comparison of the beneficial effects of the selected bioagents for promoting growth of rice as a novelty by assessing the health of the plant (e.g., gas exchange and nutritional status). Therefore, our results allow us to infer that bioagents can increase the biomass production of lowland rice plants, which is a characteristic positively correlated to crop yield (Alvarez et al. 2012). Our study under greenhouse conditions also indicated that the bioagent BRM 32109 applied only to the seed or seed-soil are the most promising combinations to be tested under field conditions, because they resulted in the greatest lowland rice development and highest biomass production among all tested bioagents (the only exception was the *T. asperellum* pool) and compared to the control (no bioagent application). Therefore, application only on the seeds should be enough to provide the beneficial effects of microorganisms on lowland rice plant development. It should also be easier and less expensive for the farmers if they need only to apply the microorganisms once during plant development. The isolate BRM 32109, identified by Martins (2015) as *Bacillus* sp. (Table 1), is characterized by the formation of endospores, resistance to adverse environmental conditions, and by presenting a multitude of antagonistic mechanisms. The bacteria of this group can colonize all plant vegetative organs, being denominated as epiphytic, endophytic, or rhizobacterium. BRM 32109 is gram-positive; produces cellulase, siderophores, and biofilms; and solubilizes phosphate.

In addition, application of BRM 32109 resulted in increased N, P, Fe, Mn, and Zn uptake by lowland rice plants compared to the control. It is also an effective antagonist of the main rice pathogens (França et al. 2015; Souza et al. 2015; Martins 2015). Therefore, proper assessment of the physiological characteristics of plants when cultivated with beneficial microorganisms can provide crucial insight into the mechanisms underlying their interactions, and those results can potentially help to identify novel strategies for sustainable crop management. Additional studies under field conditions are ongoing to verify the improvement observed in lowland rice plants after the application of rhizobacterium BRM 32109. In addition, there is a need to better understand the processes involved in bioagent-plant interactions, such as evaluating and quantifying key elements involved in the interactions. Our research opens the door for the use of bioagents in lowland plants to promote better plant growth and development.

In this sense, there are other mechanisms of growth promotion such as biochar, which is a carbon-based dusty residue obtained from phytomass pyrolysis (Marousek et al. 2016). This biochar can promote the maintenance of soil humidity, increased soil aggregation, cation exchange capacity, and carbon storage in soil, which can contribute to the mitigation of the greenhouse gas effect (Novotny et al. 2015). Although there is good information about the individual roles of PGPR, biochar, and compost in improving plant growth under normal and stress conditions, there is little information about the interactive effects of PGPR with biochar and/or compost. According to Nadeem et al. (2017), the application of biochar with PGPR and/or compost could be an effective strategy for enhancing plant growth under stress. Therefore, other studies should be done to evaluate interaction between PGPR and biochar to allow better use of these two growth promoters in crops.

Conclusion

We conducted trials under greenhouse conditions to evaluate the use of microorganism and different forms of application in lowland rice in a tropical region. Our results showed that bioagents have beneficial effects on rice plants by promoting growth (a novelty), which was done by assessing the health of the plant via evaluation of gas exchange and nutritional status. Microorganism application positively and differently affected lowland rice plant growth. The application only in the seeds was enough to provide the beneficial effects for lowland rice plant development. It is easier to adopt and the application is a low expense for farmers, who favor the method. Therefore, among the evaluated microorganisms, we selected the BRM 32109 isolate for application to seeds for future field studies, since it promoted, on average, higher dry matter biomass, gas exchange, and N, P, Fe, Mn, and Zn accumulation in leaves at 101 days after rice emergence and because dry plant biomass production for plants treated with this isolate was significantly higher than that for controls.

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